

REMARKS

Claim 36 is currently pending in this application and is in independent form. Claim 36 is currently amended. Support for this amendment can be found, for example, on pages 12 and 13 as well as in Example 1 of the specification as filed. Claim 36 is believed to be in condition for allowance. Removal of the rejection and allowance of claim 36 are respectfully requested.

Claim 36 is directed to a method of preparing an osteogenic protein fraction, extracting demineralized bone matrix with a solution of at least one chaotropic agent; removing high molecular weight proteins which exceed 300 kDA from the extract by ultrafiltration with a 300 kDA membrane to produce a lower molecular weight fraction; subjecting the lower molecular weight fraction to heparin affinity chromatography under conditions which first favor the binding and then the elution of a purified heparin affinity fraction containing the osteogenic protein fraction; subjecting the heparin affinity fraction to hydroxyapatite chromatography under conditions which first favor the binding and then the elution of a purified osteogenic protein fraction; and exchanging the purified osteogenic protein fraction into a solvent suitable for human medical use. The chaotropic agent is selected from the group consisting of urea and guanidinium salts to produce an extract.

Claim 36 is rejected under 35 U.S.C. §103(a) as being obvious over either Scott et al. (1994, The Anatomical Record 238:23-30) (hereinafter "the Scott reference") or Yoshimura et al. (1993, Biol. Pharm. Bull. 16(5):444-447) (hereinafter "the Yoshimura reference") in view of United States Patent No. 4,968,590 to Kuberasampath et. al. (hereinafter "the Kuberasampath patent").

The Scott reference is directed toward a method of isolated osteoinductive proteins from intramembraneous (IM) proteins from bones.

The Yoshimura reference is directed toward the purification of water-soluble bone-inductive protein from bovine demineralized bone matrix. The purification steps include ultrafiltration, dialysis, affinity chromatography on heparin-Sepharose and gel chromatography on Sephacryl S-200.

The Kuberasampath patent teaches hydroxyapatite chromatography after heparin affinity chromatography.

The claimed invention is not obvious over the Scott reference or the Yoshimura reference in view of the Kuperasampath patent because none of the cited prior art, either alone or in combination, teaches or suggests the claimed method of preparing an osteogenic protein fraction using a 300 kDA membrane.

The protein of the claimed invention is filtered by a 300 kDA membrane. Specifically, the claimed invention utilizes a 300 kDA membrane to remove high molecular weight proteins which exceed 300 kDA from the extract by ultrafiltration. Proteins having molecular weights of more than 300 kDA are removed at the beginning of the claimed method prior to the chromatographic steps in order to improve the yield obtained in the subsequent heparin affinity and hydroxyapatite chromatography steps. The prior art does not teach the method of the claimed invention which utilizes a 300 kDA membrane for ultrafiltration. In fact, the Scott and the Yoshimura references utilize a 100 kDA membrane to isolate proteins having weights below 100 kDA and the Kuberasampath patent does not teach removing high molecular weight portions at all. The Applicant asserts that the ultrafiltration kinetic effects associated with the use of a 100 kDA filter (as disclosed in Scott and Yoshimura) to retain protein material having a mass of above 100 kDA results in the retention of lower molecular weight material in the retentate and a resultant loss of bone morphogenic protein. The selection of a 300 kDA filter instead of a 100 kDA filter is the important distinguishing feature between the method of the present invention and the methods of Scott and Yoshimura. If anything, Scott and Yoshimura point away from the use of a 300 kDA filter.

Additionally, the Applicant respectfully presents the following Example in support of the discussion above.

EXAMPLE

The evidence pointing to the high yield of BMPs produced by the method of the claimed invention is clearly apparent when considering the empirical data generated in pilot experiments. Figure 1 is a chromatogram of the heparin-affinity procedure using the 300 kDA membrane of the invention. The unbound fraction (large arrow) is typically larger than that of the 100 kDA membrane (not shown) since more total protein passes through the larger pores of the 300 kDA membrane. The unbound (large arrow) is likewise substantially reduced following removal of collagenous peptides, and the osteogenic fraction (small arrow) contains four times more protein than that produced in the method of Kuberasampath, and an estimated two-fold more protein when using a 100 kDA membrane.

The advantage gained using the method of the present invention can be clearly distinguished by the hydroxyapatite chromatography data shown in Figure 2. The chromatograms shown in Figure 2a and Figure 2b, which are for two different batches of BMPs, show the larger relative size of the BMP fraction (large arrow) compared to the size of the unbound fraction (smaller arrow). A direct comparison with the chromatographic profiles of Kuberasampath (Figure 2c) shows a much larger relative size of the unbound fraction, indicating that the present invention leads to greatly enhanced recovery of osteogenic fraction. Using ELISA assay and antibodies for BMP-2 for four other experiments, the Applicant has found that a total final recovery of an average of 41% of the total BMP in the original raw material (Table 1) is recovered into the final product, confirming the high yield capabilities of the process. When applied to single donor bone technology (Figure 1), yields as high as 75% have been achieved.

Table 1. Yield data of empirical experiments using 300 kDA membrane.

| Lot # | estimated dry DBM kg | estimated total hBMP-2 @25 ng/g DBM (ng) | estimated natural abundance hBMP-2 @ 25ng/g DBM | total hBMP complex (mg) | total hBMP-2 purified ng | percent yield hBMP-2 from estimated total |
|----------------|-------------------------------------|---|--|--|---|--|
| Lot 01 - 2006 | 1.5 | 22500.0 | 0.00000250% | 51 | 12236 | 54.4% |
| Lot 02 - 2006 | 3.0 | 45000.0 | 0.00000250% | 62.608 | 11774 | 26.2% |
| Lot 03 - 2006 | 1.7 | 24750.0 | 0.00000250% | 80.35 | 9935 | 40.1% |
| Lot 04 - 2006 | 1.5 | 22500.0 | 0.00000250% | 73.695 | 10336 | 45.9% |
| <i>average</i> | | | | | | 41.7% |

Rapid processing is also important for the collection of a high activity of BMP. However, the use of a 100 kDA membrane, as in Scott and Yoshimura, requires:

- (a) higher transmembrane pressures;
- (b) higher throughput volume of the entire system, i.e., higher number of passes of the extract over the membrane;
- (c) a longer period of time to complete the ultrafiltration step; and
- (d) interventions to minimize fouling of the column by collagens and collagen aggregates, which would affect the flux through the membrane which reduces the biological activity of the final product because:
 - (1) higher pressures result in higher shear forces exerted on the BMP protein,

and therefore higher rates of denaturation can be expected;

- (2) longer retention times in the system increase the contact time of BMP and extracted proteases, leading to increased proteolysis of BMP by extracted proteases; and
- (3) smaller effective pore size and greater fouling reduce the passage of the 30 kDA BMP through the membrane for a given amount of extract passes.

All of these disadvantages are overcome by the use of a 300 kDA membrane.

Because ultrafiltration with a 300 kDA membrane is not the same as filtering proteins with a 100 kDA membrane, as described by the Scott and Yoshimura references, the claimed invention is not taught or suggested by the prior art.

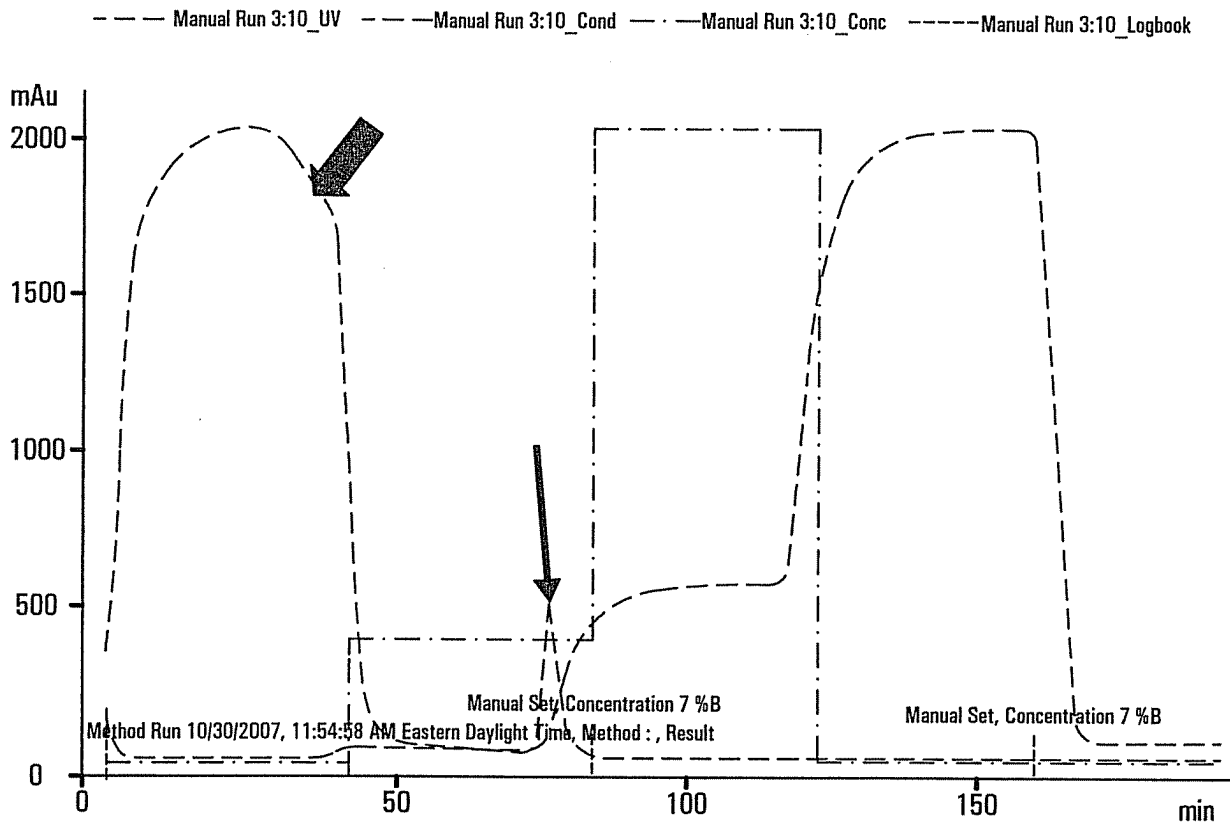


FIG 1

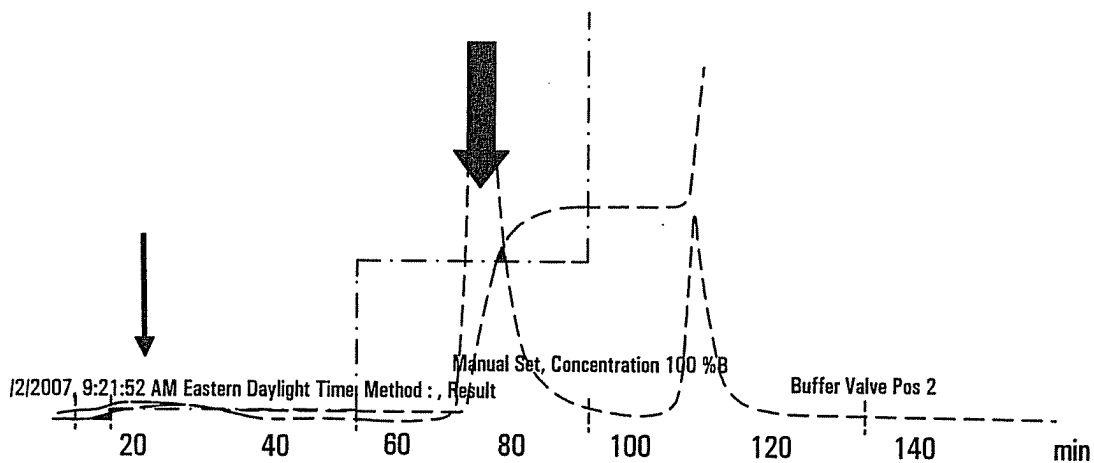


FIG 2a

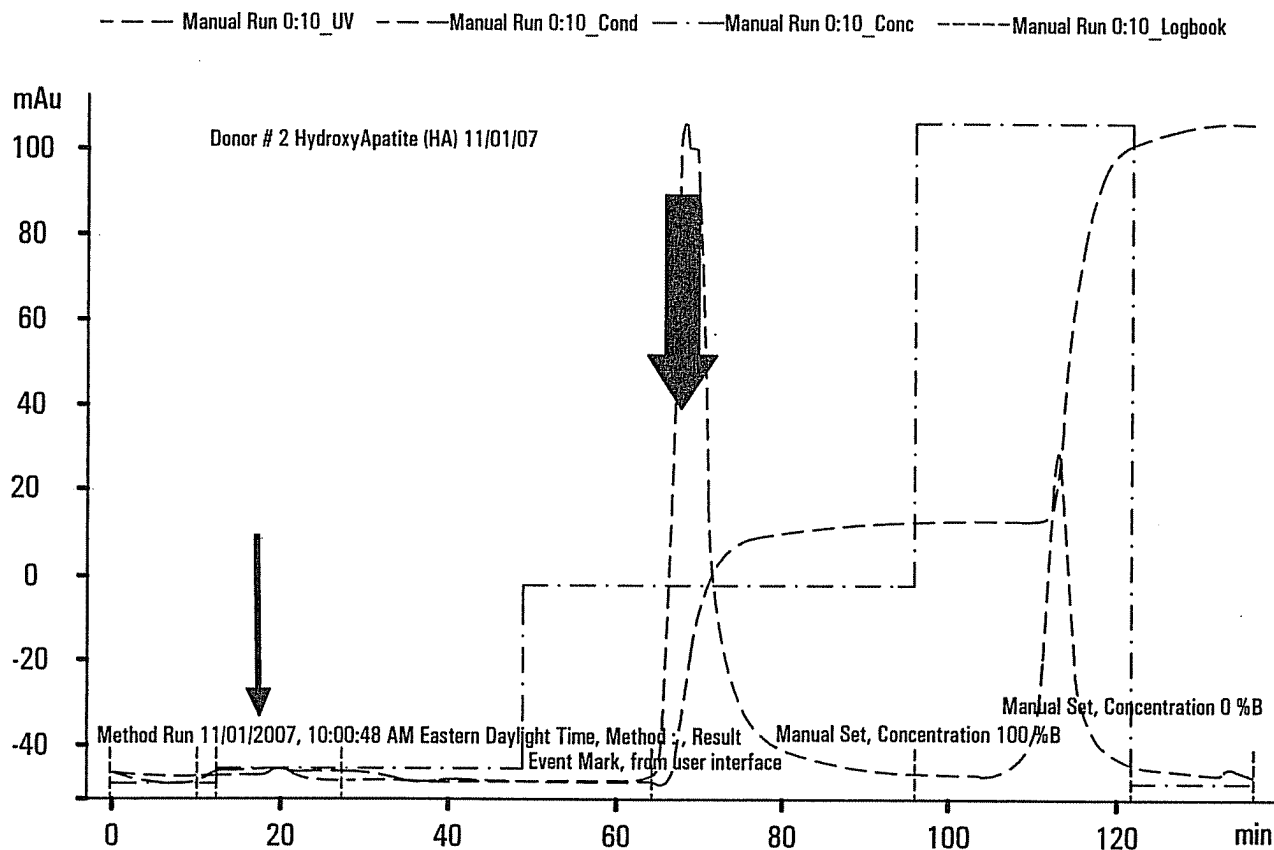


FIG 2b

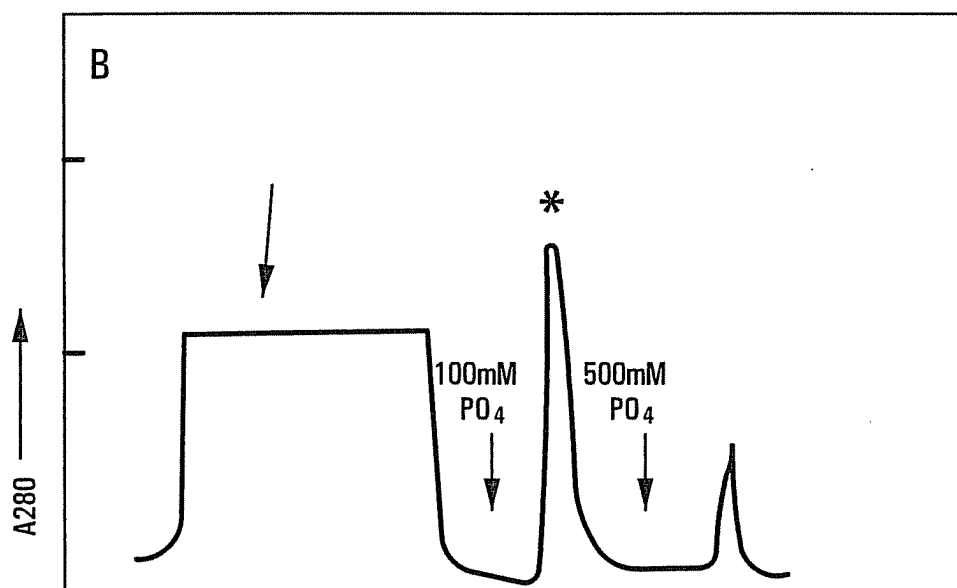


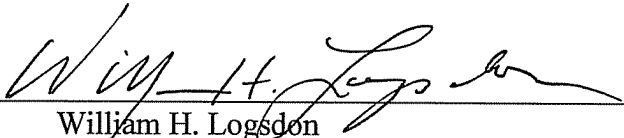
FIG 2c

Application No. 10/518,723
Amendment dated August 28, 2008
In Reply to Office Action of April 28, 2008
Attorney Docket No. 2226-045890

CONCLUSION

For the foregoing reasons, Applicant submits that Scott and Yoshimura neither teach nor suggest the use of a 300 kDA filter in order to isolate the osteogenic protein fraction of the claimed invention and that the method of the present invention is accordingly both novel and nonobvious in view of the disclosures of Kuberasampath, Scott and Yoshimura. Reconsideration of the rejections and allowance of claim 36 are respectfully requested.

Respectfully submitted,
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